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13. ABSTRACT (Maximum 200 words)

The project objective was to determine the degree of plant stress detectable with remote fluorescence measurements in response to application of the stressors: salinity, drought, herbicide, organophosphate-based pesticide, and trampling. All experiments were conducted with *Phragmites australis* (common reed), a weedy wetland grass common throughout the mid-Atlantic region. The degree of stress was quantified through measured changes in photosynthetic rate, leaf pigment concentrations, stomatal conductance and leaf xylem pressure potential. The trampling pesticide and trampling experiments were inconclusive, there was no evident change in fluorescence. For salinity and herbicide treatment, induced stress was detectable with measurements of fluorescence emission spectra. Stress was evident at 10 ppt salinity and higher, and within 24 hours of herbicide application. Relative to control plants there were significant decreases in fluorescence emission intensity across wavelengths from 678 to 765 nm. Additional preliminary experiments with drought stress revealed significant changes in fluorescence within four days and corresponded to reductions in water relations parameters. For these experiments, fluorescence decreases corresponded to significant decreases in all physiological parameters, except leaf pigment concentrations. For most experiments, decreases in fluorescence were measured before the plants appeared to be visibly stressed. These preliminary results indicate that analysis of fluorescence emission spectra may accurately indicate the onset of stress in plants.

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Enclosure 1

FINAL PROGRESS REPORT:

"Fluorescence Remote Sensing and Plant Stress"

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Statement of the Problem Studied

The objective of this research proposal was to apply various stressors, quantify the degree of stress via physiological measurements, and determine the degree of stress that is detectable through remote fluorescence measurements. Stressors included salinity, herbicide, organophosphate-based pesticide (as a surrogate nerve agent), and disturbance / compaction (i.e. simulated trampling). A preliminary experiment with drought stress was also conducted. Physiological measurements included photosynthetic properties, chlorophyll contents, and water relations. Fluorescence was quantified through non-imaging spectroscopic techniques. The research was conducted in cooperation with the US Army ERDC laboratory, Alexandria, Virginia.

Research Approach

All experiments were conducted using the brackish wetland grass, *Phragmites australis* (common reed). *Phragmites* rhizomes were collected in the field and returned to the laboratory and potted for use in the glasshouse experiments. A minimum of 10 potted plants was used for each experiment.

Stress was induced in weekly increments and stress measurements (both physiological parameters and fluorescence) were conducted 1, 2, and 5 days after treatment. For salt stress, soil salinity was be increased each week by the addition of salt solutions of 2, 5, 10, 15, and 20 ppt. Herbicide stress was induced by the application of a commercially available broad spectrum herbicide (Roundup). The herbicide was applied at concentrations of 50 and 100% of the recommended

concentration. An identical procedure was followed for a commercially available insecticide (Malathion).

Disturbance stress experiments followed a slightly different procedure. Plants were flattened with plywood squares (0.5x0.5 m) for approximately eight hours. Stress measurements were conducted at 4, 8, 12, and 24 hours after stress treatment.

A preliminary experiment with drought stress compared changes in the physiological parameters and fluorescence spectra between control and drought stressed plants. Drought stress was induced by withholding water. The plant physiological parameters and fluorescence emission spectra were then monitored at daily intervals until stress was visually evident.

Physiological measurements included maximum rate of photosynthesis, stomatal conductance to water vapor diffusion, xylem pressure potential, and chlorophyll concentration. Photosynthesis and stomatal conductance were measured with a portable infrared gas analyzer (LI-COR 6400) and xylem pressure potential with a Scholander pressure chamber (PMS 650) using standard protocols. Relative chlorophyll concentrations were determined with a Minolta SPAD 502 meter. Intrinsic, steady-state fluorescence, based on excitation with 455 and 680 nm light, was measured for all vegetation samples by using a JY Horiba Fluorolog 3 luminescence spectrometer. All fluorescence measurements were recorded *in vivo* and were non-destructive to the plants.

Summary of Results

Salinity Stress: Whole plant physiological measurements indicated that *Phragmites* was stressed at 5 ppt salinity and beyond (Fig. 1). There was a significant decrease in stomatal conductance at 5 ppt. This was followed by a significant decrease in photosynthesis (A_{Net}) at 10 ppt. Xylem pressure potential differences between salt treated and control plants were significant at 15 ppt (Fig. 1). In contrast, there were no significant changes in chlorophyll concentration as salinity increased (Fig. 2). However, chlorophyll concentration for the salt treated plants was slightly elevated relative to the non-stressed controls.

A visible difference between salinity stressed and control plants became apparent between 10 and 15 ppt. Fluorescence measurements indicated a significant decline in spectral emission intensity at 10 ppt and beyond when excited with 455 nm (Fig. 3). Differences in fluorescence intensity were most evident between the peaks at 680 and 735 nm. In comparison, there was a significant decline in spectral emission intensity at 15 ppt when excited with 680 nm (Fig. 3). The decline was most evident 700 to 730 nm.

An examination of changes in fluorescence emission intensity at 15 ppt salinity showed a steady, significant decline relative to control plants from day 1 through day 5 of treatment. The decline was most evident between the peaks at 680 and 735 nm when plants were excited with 455 nm (Fig. 4). Declines were also evident and significant for plants excited with 680 nm (Fig. 4). The most sensitive wavelength region was 700 to 740 nm.

Conclusion: Salinity stress for *Phragmites* was detectable with changes in fluorescence emission intensity. These changes were detectable before the plants were visibly stressed (i.e. wilted, senescent leaves, etc.).

Herbicide treatment: Plant response to herbicide (Roundup) was both rapid and variable. For all experiments, visible effects were evident within 24 hrs of treatment, regardless of herbicide concentration. Further, response was so rapid that meaningful physiological measurements were not possible. For half strength treatment, fluorescence emission intensity was slightly elevated relative to control plants one day after treatment and greatly elevated at three and five days after treatment when excited with 455 nm (Fig. 5). Differences in fluorescence were evident from 630 through 800 nm but were most pronounced between the peaks of 680 and 740 nm. A relative flattening of the spectral response curve on day 5 indicates a dysfunctional photosystem for the light reactions of photosynthesis. Similar changes were evident for stress plants excited with 680 nm (Fig. 5). Increases relative to control plants were evident from 700 to 800 nm, but these were most pronounced from 700 to 740 nm.

For the full strength herbicide experiment, plants were obviously dead three days after treatment. Relative to control plants, there were significant declines on day one and day two after treatment in fluorescence emission intensity when excited with 455 or 680 nm (Fig. 6).

Conclusion: Stress from application of herbicide to *Phragmites* was clearly detectable, which corresponded to visible evidence of stress. Perhaps much lower concentrations (e.g. $\leq$10%) may be detectable with fluorescence measurements before the plants are visibly affected.

Pesticide treatment: No changes in physiological parameters were detected regardless of the concentration of pesticide when plants were followed for up to eight days after treatment. However, when excited with 455 nm, there were slight variations in fluorescence emission intensity relative to controls (Fig 7). Days 1,2 and 8 decreased, whereas days 3 and 6 increased. Similarly, when excited with 680 nm, fluorescence increased on day 1 and 2 and decreased relative to day 8 (Fig. 7).

Conclusion: The application of pesticide, even at full concentration, did not lead to detectable stress in *Phragmites*.

Trampling treatment: No changes in physiological parameters or fluorescence emission intensity between treated and control plants were detected regardless of the degree of trampling.

Drought treatment: This preliminary experiment compared drought stressed *Phragmites* to well watered controls. There were no differences in physiological parameters until plants had not been watered for four days. From day 4 through day 6, *Phragmites* xylem pressure potential decreased continually and remained significantly lower relative to the control plants (Fig. 8). By day 6, the above ground portions of the drought stressed plants were dead. Differences in chlorophyll concentration were not apparent until day 6. When excited with 455 nm, the fluorescence emission intensity significantly differed from controls on day 5 and beyond (Fig. 9). Fluoresence emission intensity was greater for stressed plants on day 5, but lower on days 6 and 7. A general flattening of the spectral peaks indicated a dysfunctional photosystem. Nearly identical results occurred when plants were excited with 680 nm (Fig. 9).

Conclusion: The onset of drought stress in *Phragmites* is relatively sudden and was detectable with measurement fluorescence emission intensity at about the same time that the effects were visually apparent.

Publications and Reports: No papers have been published or presented at meetings and not manuscripts have been written.

Participating Scientific Personnel:

Dr. Donald R. Young, Dept. of Biology, VCU - principal investigator

Ms. Maria L. Mood, Dept. of Biology, VCU - lab technician

Dr. John Anderson, US Army ERDC Laboratory, Alexandria, VA - project advisor

(no degrees were awarded to project personnel)

Inventions: No inventions have been developed based on any facet of the project.

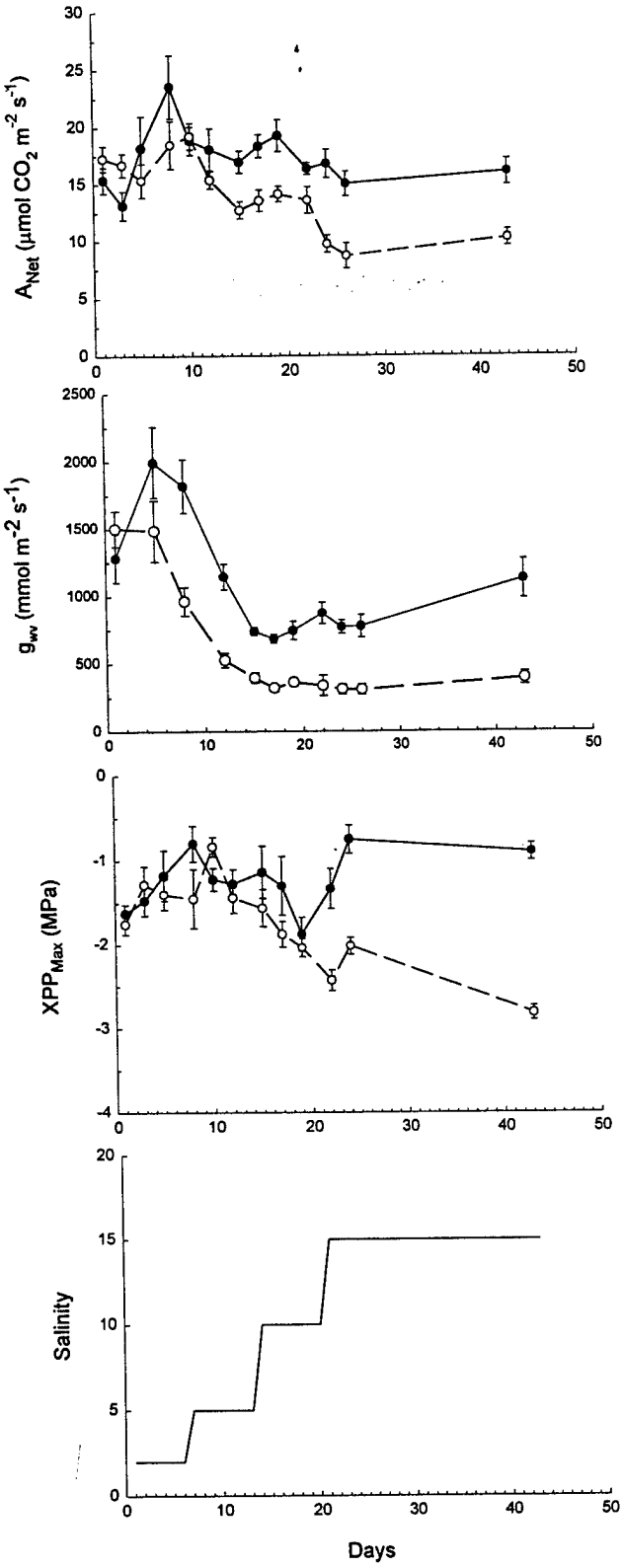

Figure 1. Net photosynthesis (A_{Net}), stomatal conductance (g_{WV}), xylem pressure potential (XPP) and salinity (ppt) treatment for *Phragmites australis* treated (open symbols) and control (closed symbols) plants. Values are means ± standard errors.

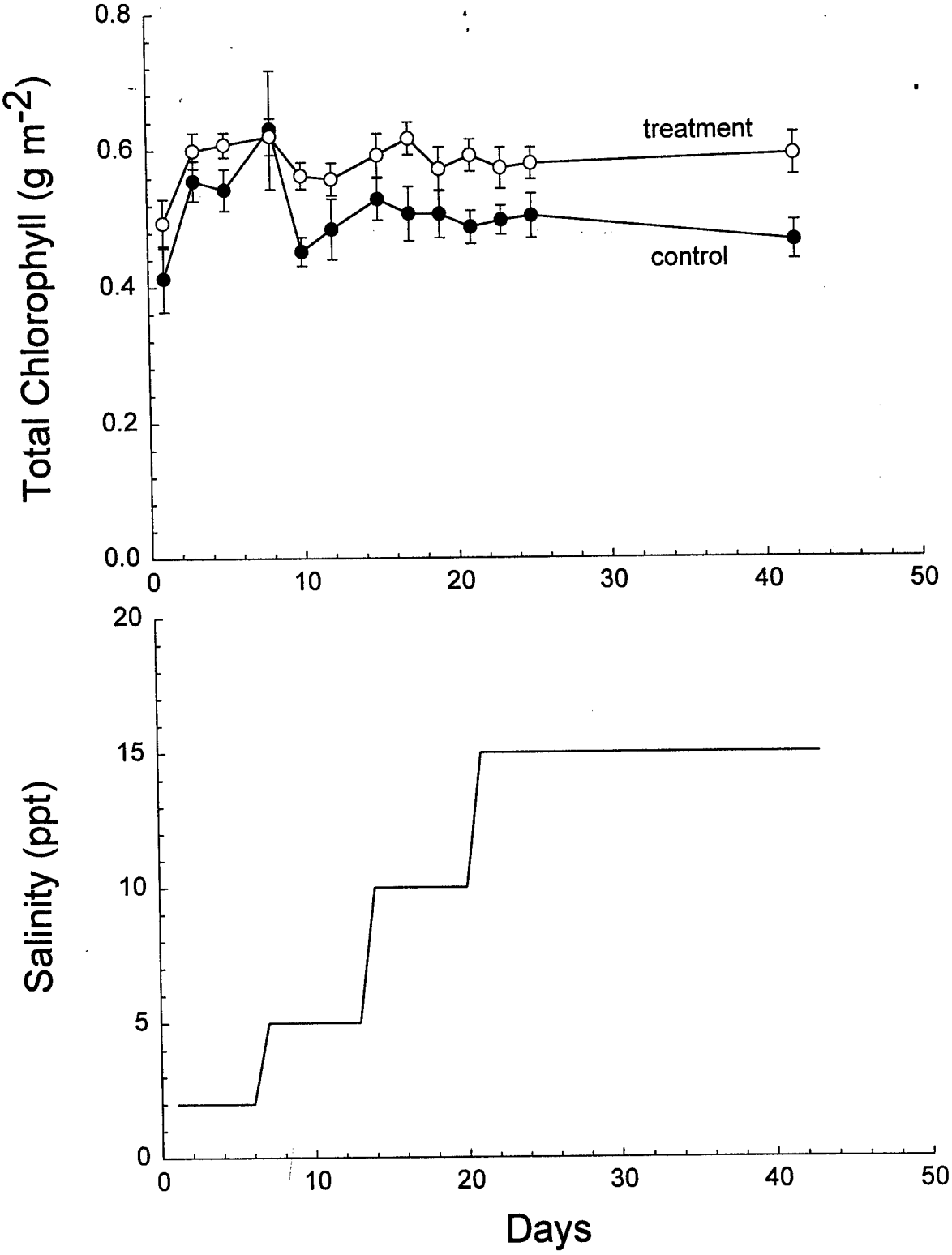

Figure 2. Estimated chlorophyll content and salinity treatment through time for *Phragmites australis* treatment and control plants. Values are means ± standard errors.

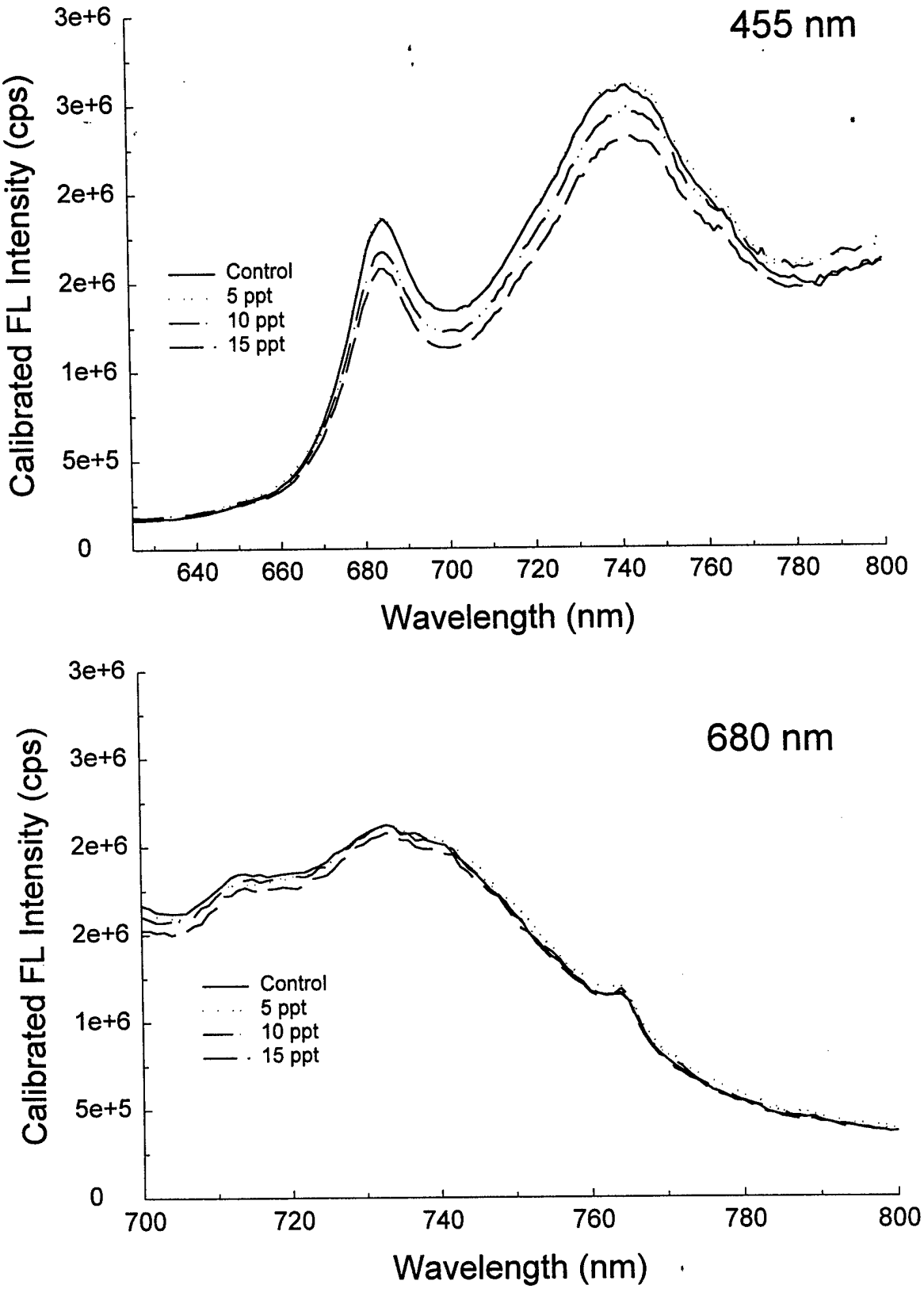

Figure 3. Fluorescence emission intensity for *Phragmites australis* control and 5, 10 and 15 ppt salinity treated plants excited with 455 and 680 nm.

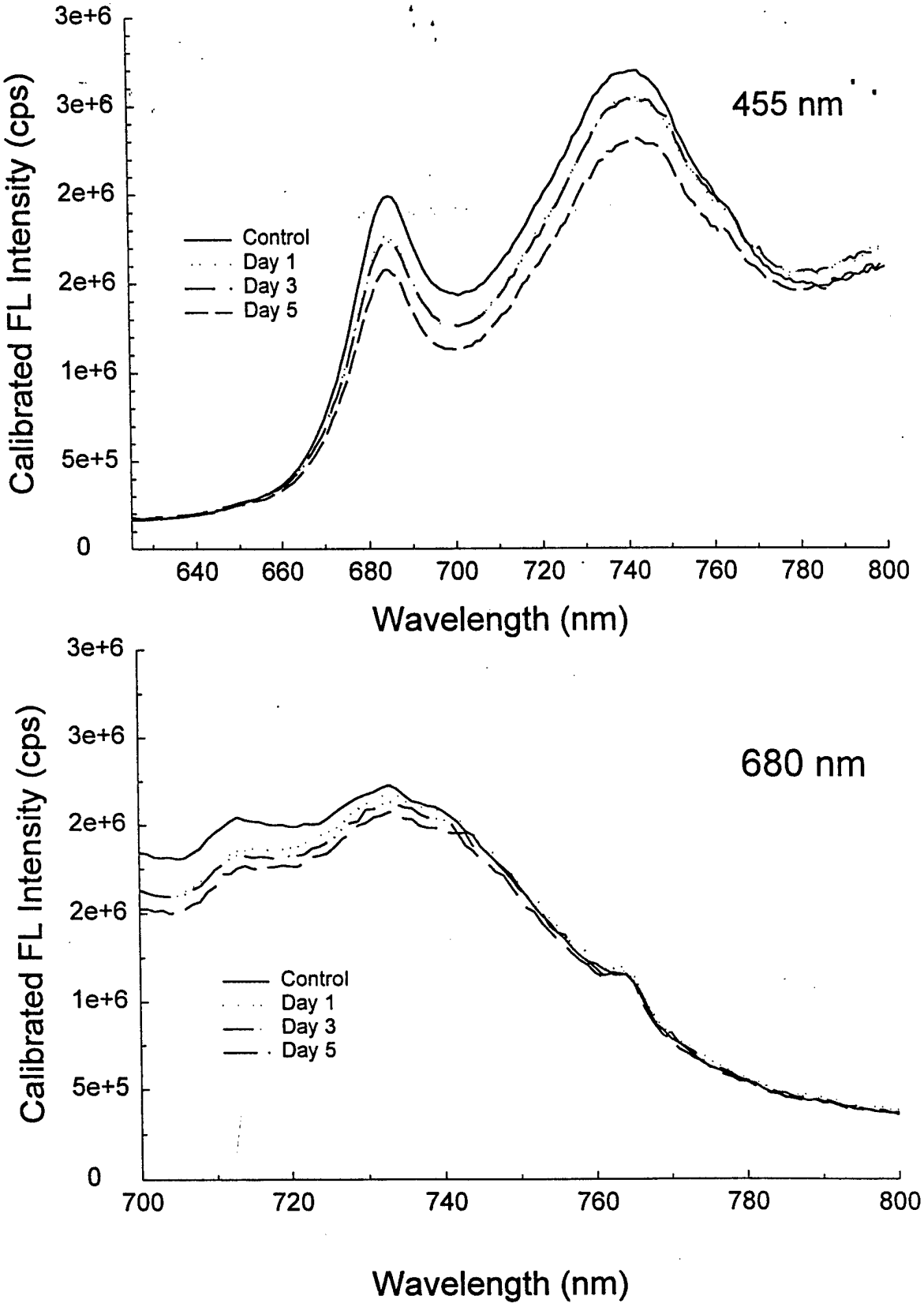

Figure 4. Fluorescence emission intensity for *Phragmites australis* excited at 455 and 680nm after 1, 3 and 5 days of 15 ppt salinity treatment. Controls received no salt.

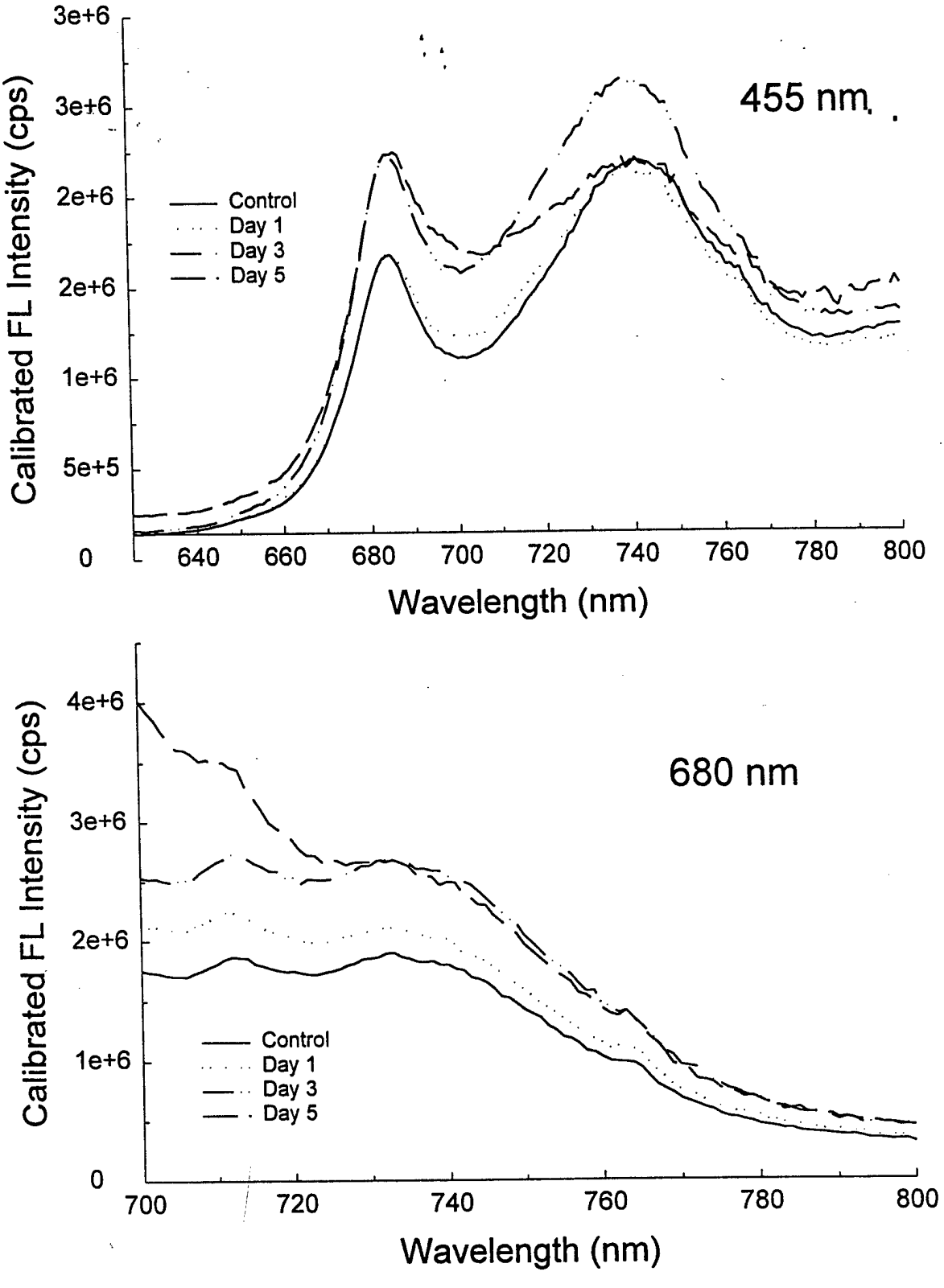

Figure 5. Fluorescence emission intensity for *Phragmites australis* excited at 455 and 680nm after 1, 3 and 5 days of half-strength herbicide (Roundup) treatment. Controls received no herbicide.

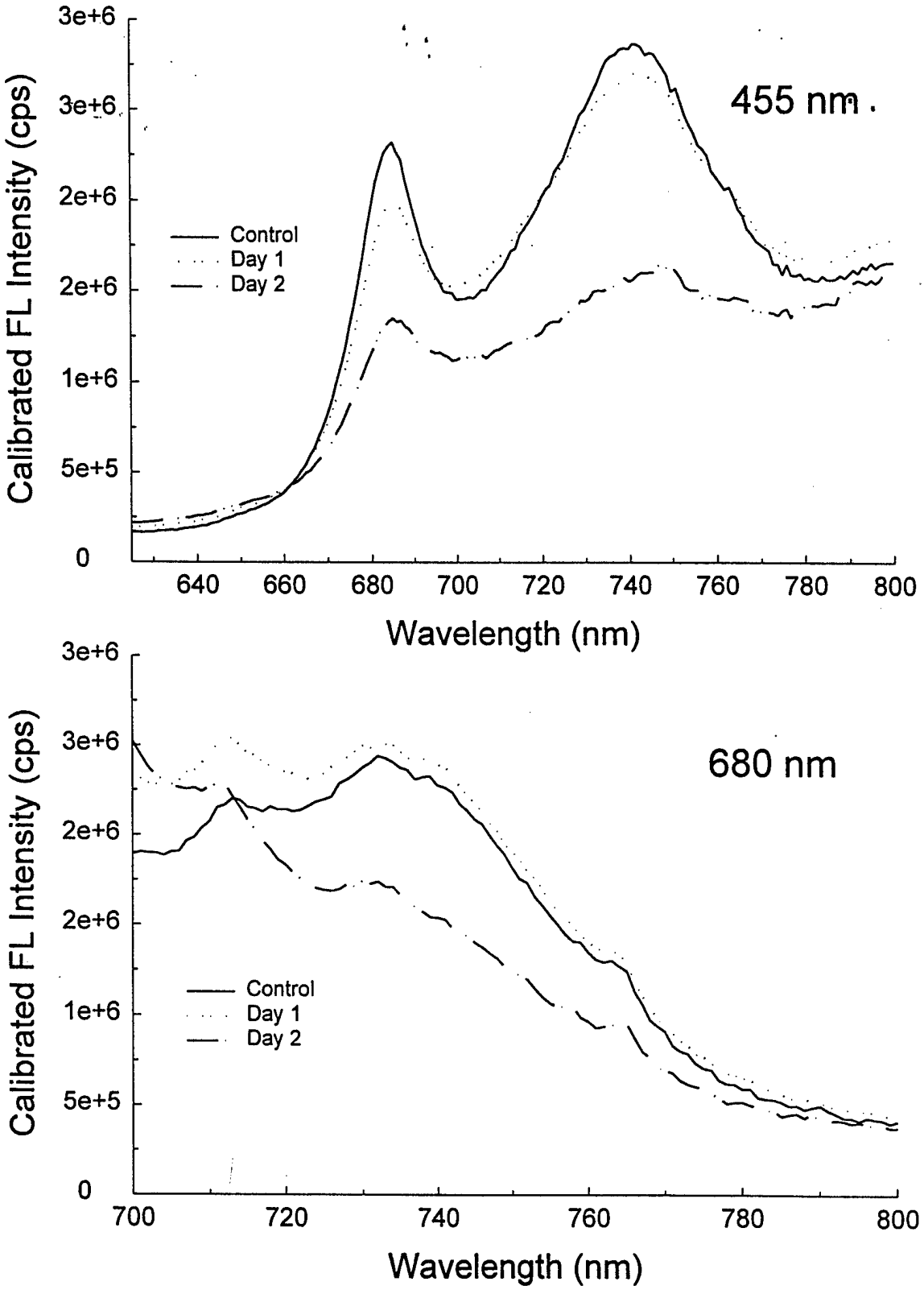

Figure 6. Fluorescence emission intensity for *Phragmites australis* excited at 455 and 680nm 1 and 2 days after full-strength herbicide (Roundup) treatment. Controls received no herbicide.

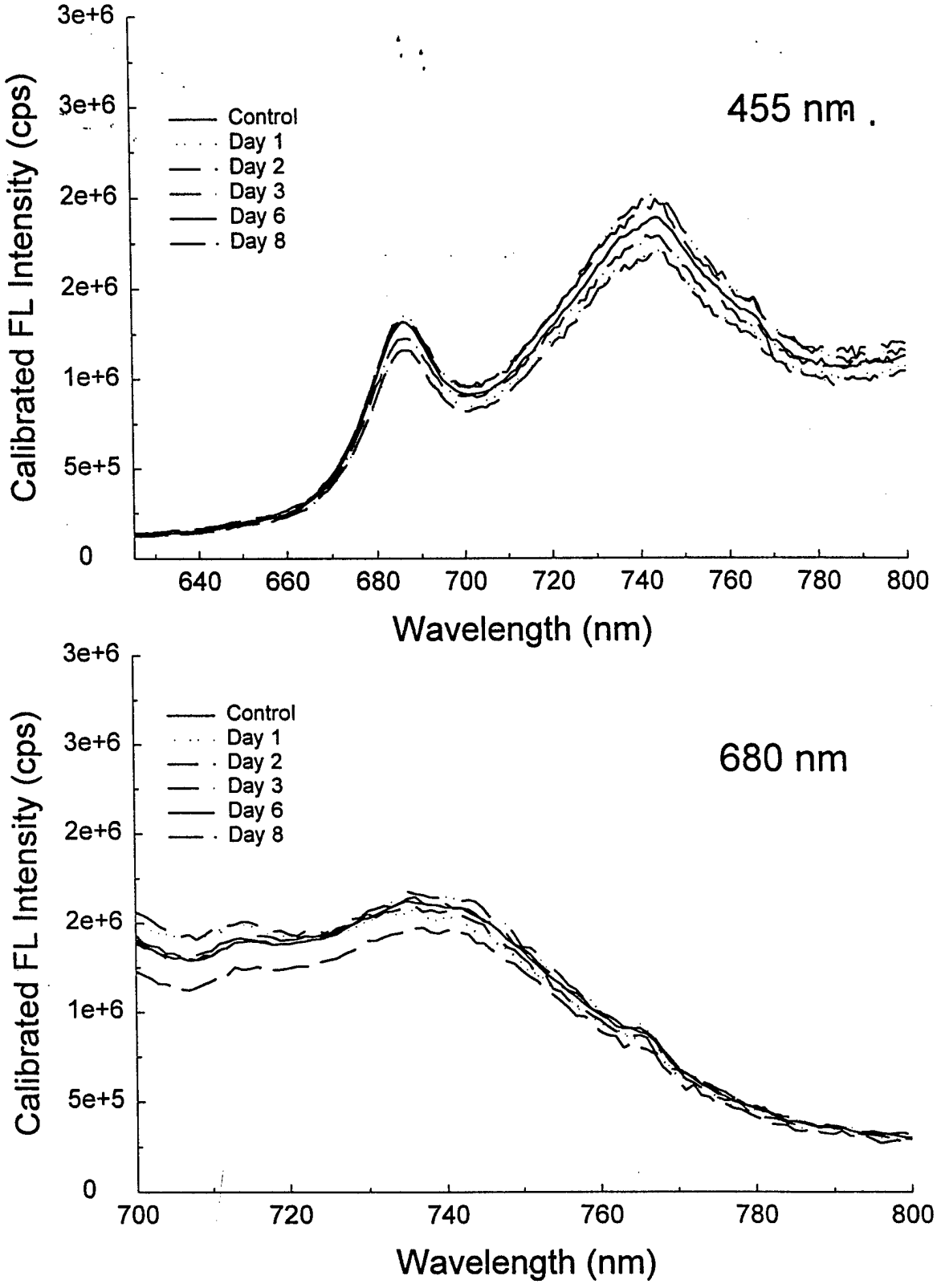

Figure 7. Fluorescence emission intensity for *Phragmites australis* excited at 455 and 680nm day 1 through day 8 after full-strength pesticide (Malathion) treatment. Controls received no pesticide.

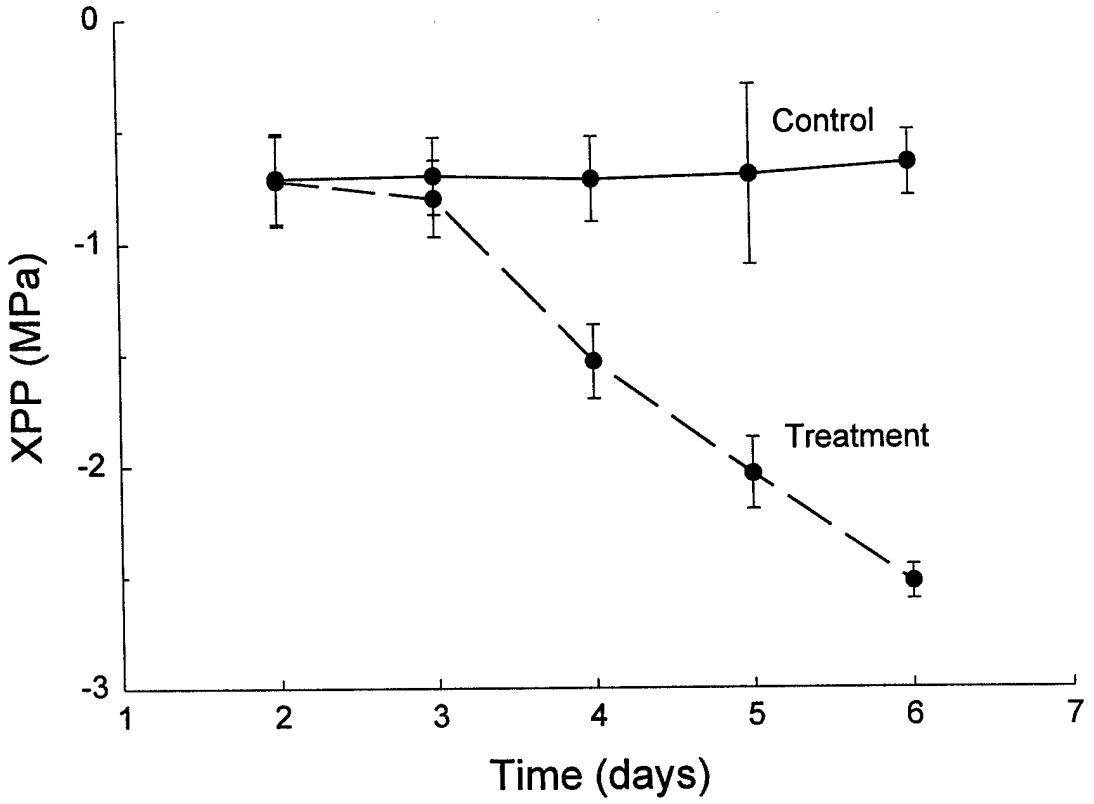

Figure 8. Xylem pressure potential through time for droughted and well-watered (control) *Phragmites australis.* Values are means ± standard errors.

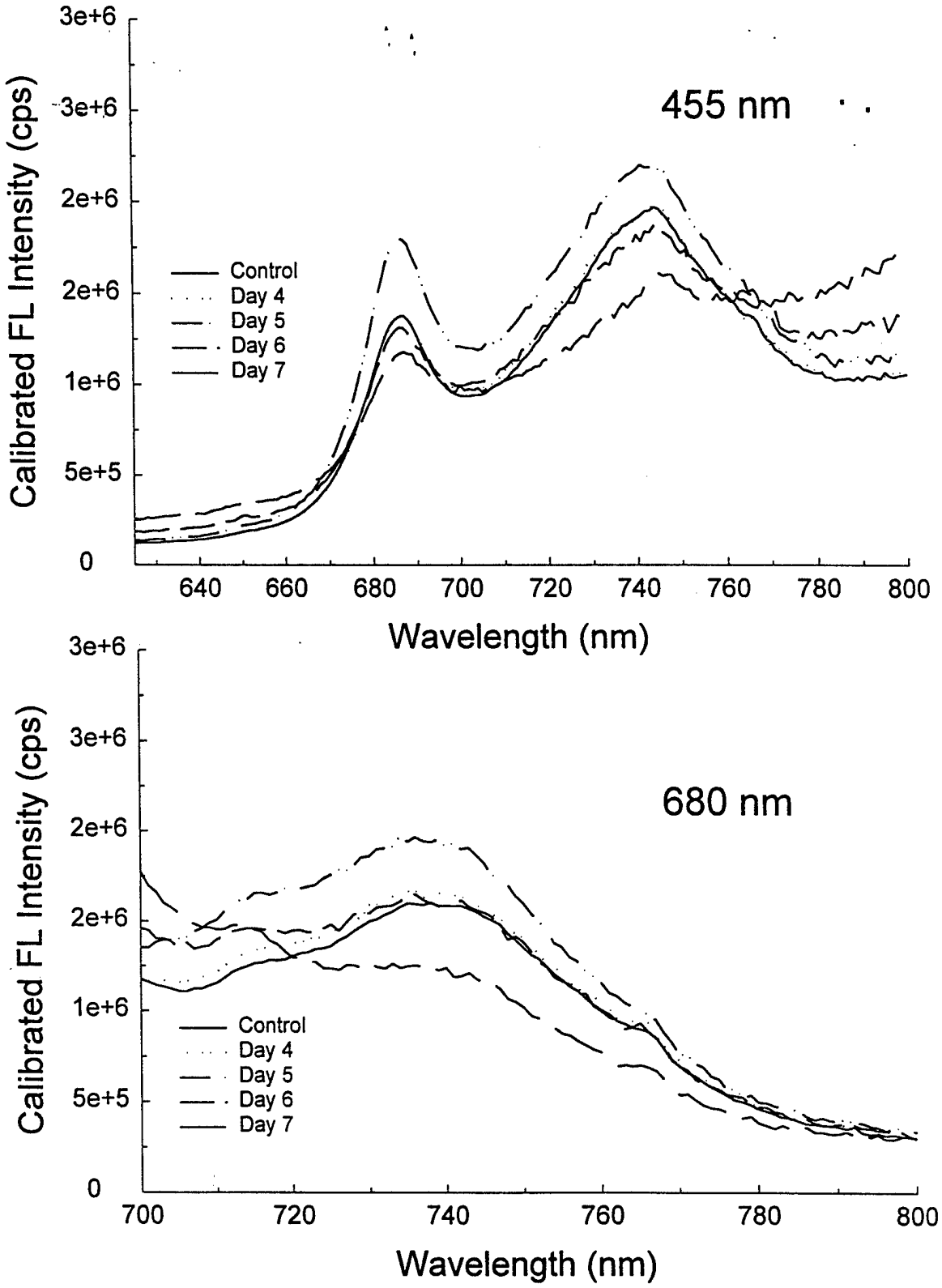

Figure 9. Fluorescence emission intensity for *Phragmites australis* excited at 455 and 680nm day 1 through day 7 of drought treatment. Controls were well-watered.